

Determination of Carbon-14 in Solutions of C^{14} -Labeled Materials by Means of a Proportional Counter¹

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A study is reported of the determination of carbon-14 in C^{14} -labeled substances by counting, with a proportional gas-flow counter, the radioactive disintegrations arising from a material that is dissolved in a suitable solvent. A number of solvents were found to be satisfactory. Formamide is recommended for the assay of carbohydrates and other materials with which it is a compatible solvent. Ethylene glycol is suitable for the assay of highly alkaline substances, and, in conjunction with sequestering agents, for the assay of alkaline earth carbonates. Other useful solvents are dimethyl formamide and 90-percent phosphoric acid. The counting efficiency in different solvents was found to be inversely proportional to the density of the solution and highly reproducible. The study shows that solutions of C^{14} -labeled materials can be counted easily and precisely with a proportional counter.

1. Introduction

Relatively little work has been done on the counting with proportional counters of C^{14} -labeled compounds in solution [1, p. 101].² Direct measurement of the radioactivity of solid sources is complicated by variations in self-absorption, backscattering, and foreshadowing, and (in counting on plastic backings) by the presence of static charges that distort the field. These variations do not occur with solutions, which are strictly reproducible, and have little tendency to hold static charges. Other complications arise, however, and one cannot obtain satisfactory assays with most solvents. In a prior publication from the Bureau the use of formamide solutions for the determination of carbon-14 in labeled materials was described [2]. The present paper presents further studies of the method and of the application of other solvents.

2. Discussion of the Experimental Technique

2.1. Counting Apparatus

Figure 1 illustrates a typical proportional gas-flow counter. The instrument is designed so that a continuous stream of a "self-quenching" gas flows at about atmospheric pressure through the counting chamber, which contains the material to be analyzed. Radiation from the material under analysis produces ionization in the chamber; this leads to a momentary discharge across the high-voltage field maintained between the wall of the chamber and a wire anode. The change in potential caused by the discharge is converted into a current pulse that activates a recording mechanism by means of a vacuum-tube amplifier [3, p. 29]. Instruments of this type are available commercially and are readily adapted for assay of liquid samples. They are much more stable and sensitive than thin-window Geiger counters; hence they are particularly suitable for measurement

of low-energy beta radiation, such as that from carbon-14.

To adapt commercially available proportional counters for the assay of liquid samples it is necessary to fit them with a suitable cell to hold the liquid. Figure 2 shows the stainless-steel cell used in the present study. It has an inside diameter of 36 mm and a depth of 1.1 mm. When full, it holds 1.1 ml of solution. In the analysis approximately 1 ml of solution is placed in the cell. The amount is not critical because even 0.5 ml gives an "infinitely thick" layer of the solution with respect to penetra-

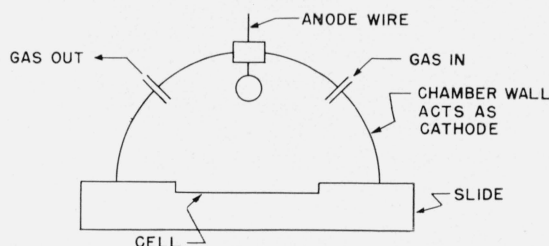


FIGURE 1. Diagram of proportional gas-flow counter.

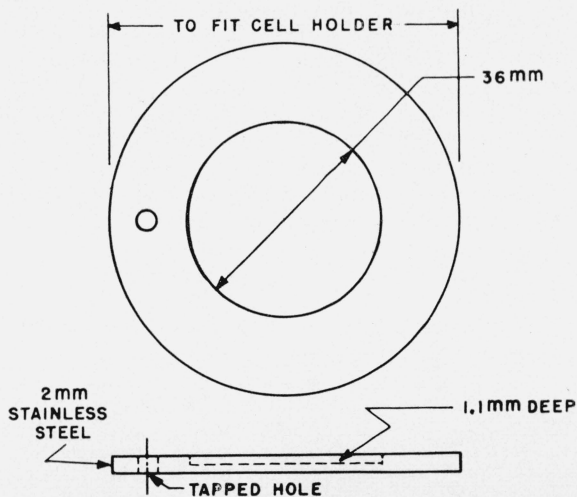


FIGURE 2. Cell for counting solutions.

¹ Part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the Atomic Energy Commission. This paper is based on the work described in a previous AEC report.

² Figures in brackets indicate the literature references at the end of this paper.

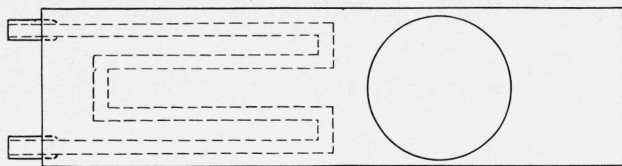


FIGURE 3. Top view of water-cooled slide.

tion of the radiation from carbon-14. The cell is handled without contamination by use of a bolt that fits the tapped hole and serves as a removable handle.

In instruments in which the counting chamber is surrounded by electronic tubes and other parts that liberate heat, the temperature of the solution rises with long counting periods. The rise in temperature causes evaporation of volatile constituents in the solution under analysis, and this leads to erroneous results. This difficulty may be overcome by the use of a cooling device. In the present study the counter slide of a commercial instrument³ was drilled as shown in figure 3, and a stream of cold water was circulated through the passage. With instruments in which the counting chamber remains at room temperature, a cooling device is not necessary.

2.2. Solvent

A satisfactory solvent for counting of C^{14} -labeled materials in solution by means of a proportional counter should have the following characteristics:

1. It should be an adequate solvent for the material to be analyzed.
2. Its vapor pressure should be low, to avoid a change in the concentration of the solute with time.
3. Its vapors should not form a conducting path across the high-voltage wire insulation.
4. It must have little or no tendency to creep over the edge of the cell.

In the present study, many liquids were tried. Water caused insulation breakdown; monomethyl and monoethyl ethers of ethylene glycol (Methyl Cellosolve and Ethyl Cellosolve, respectively) evaporated slightly and also showed a marked tendency to creep; 75-percent sulfuric acid was found to be useful, but on long standing it crept slightly; 90-percent phosphoric acid was fairly satisfactory whereas purified formamide, dimethyl formamide,⁴ and ethylene glycol gave excellent results. Solutions of acetamide in formamide were found to be satisfactory, and undoubtedly other solvents could be used. Ordinarily, commercial formamide contains sufficient ammonia to cause insulation breakdown in the counter. For use as a counting liquid it should be purified by drying with anhydrous sodium sulfate and distilling under reduced pressure. The middle, ammonia-free fraction is satisfactory, for counting purposes, and can be kept for months without deterioration.

The amount of water that can be present in the solvent without interference depends, in part, on the

operating conditions, especially the temperature, the rate of gas flow, and the design of the counter. With an instrument in which the counting chamber is not enclosed in a case with the electronic mechanism, up to 10 percent of water can be present in a formamide solution without interference. With the water-cooled slide shown in figure 3, satisfactory results were obtained with formamide solutions containing up to 15 percent of water, but ordinarily the water content should be kept as low as possible to minimize error due to evaporation.

2.3. Analytical Procedures

The solution to be assayed can be prepared by any convenient method. Readily soluble materials are dissolved directly in the counting liquid. Aqueous solutions are prepared for assay by taking a definite volume (up to 0.1 ml) in a mixing pipet and then adjusting the volume to 1 ml with formamide or other suitable solvent. The separation of insoluble precipitates or a second liquid phase must be avoided. To minimize errors that might arise from adsorption of the radioactive material on the cell or from concentration of the sample on the surface of the counting liquid, highly active materials are diluted with a suitable quantity of the corresponding nonradioactive material when the solution is prepared for counting. After the solution has been placed in the counter, the system is thoroughly flushed with the counting gas, and the count is taken. For maximum precision, background determinations are made before and after each measurement.

2.4. Precision of Measurements

Tables 1 and 2 show the reproducibility of measurements made on a C^{14} -labeled sugar in formamide and in ethylene glycol, using three proportional counters differing slightly in counting efficiency. The counts for the two solutions are not directly comparable because the solutions differ in concentration and density. In accordance with the conventional statistical treatment [3, p. 43] of the disintegration rate of a radioactive substance, one would expect a standard deviation of ± 309 for 95,000 counts. The standard deviations obtained for the experimental data are only slightly higher than this value. Thus C^{14} -labeled compounds can be reproducibly counted in solutions of either formamide or ethylene glycol.

The count for the formamide solution with instrument No. 3 increased slightly as the measurements progressed. A change of this character might arise from an instrument drift, or from evaporation of the solvent with a corresponding increase in the concentration of the solute. Instrument 3 was cooled by circulating water at about 25° C, whereas instruments 1 and 2 were cooled with water at about 15° C. Thus it seems possible that the increase was caused by evaporation of the solvent. The increase was small and would be insignificant with a normal counting period.

³ Nuclear Measurements Corporation, Indianapolis, Ind., model PC-1.

⁴ Suggested by W. H. Smith of the Bureau.

TABLE 1. Reproducibility of successive 8-min counts of D-galactose-1-C¹⁴ in formamide solution ^a

Proportional counter 1		Proportional counter 2		Proportional counter 3	
Count	Deviation from mean	Count	Deviation from mean	Count	Deviation from mean
94,144	+412	94,886	+512	89,678	-1,223
93,952	+220	94,349	-25	90,014	-887
93,542	-190	94,170	-204	89,897	-1,004
94,054	+322	94,272	-102	90,868	-33
93,542	-190	93,900	-474	90,401	-500
94,413	+681	94,566	+192	91,047	+146
93,402	-330	94,540	+166	90,313	-588
93,325	-407	95,002	+628	90,677	-224
93,542	-190	94,323	-51	91,000	+99
93,926	+194	95,116	+742	90,773	-128
93,632	-100	94,382	+8	91,851	+950
93,645	-87	94,067	-307	90,579	-322
93,389	-343	94,362	-12	91,178	+277
93,542	-190	94,566	+192	90,942	+41
93,606	-126	94,221	-153	91,903	+1,002
93,414	-318	94,349	-25	90,774	-127
93,926	+194	94,578	+204	91,277	+376
93,555	-177	94,285	-89	91,121	+220
94,643	+911	94,170	-204	92,055	+1,154
93,696	-36	94,131	-243	91,672	+771
93,773	+41	93,798	-576	-----	-----
93,286	-446	94,208	-166	-----	-----
93,964	+232	94,221	-153	-----	-----
93,978	+246	94,387	+13	-----	-----
93,414	-318	94,515	+141	-----	-----
Avg. 93,732	-----	94,374	-----	90,901	-----
Standard deviation	339	-----	301	-----	640

^a The solution contained 0.415 mg of D-galactose-1-C¹⁴ per milliliter.

2.5 Calibration

To convert the observed counts per second (cps) to the absolute disintegration rate (dps), or to microcuries per milliliter of solution, it is necessary to standardize the procedure by counting a solution containing a C¹⁴-labeled substance of known radioactivity under conditions comparable to those used in the analysis. In the present study several materials were used for standardization. The radioactivity was determined by oxidizing the material [1, p. 92], transferring the carbon dioxide to an ionization chamber, and comparing the drift rate of a vibrating-reed electrometer with the drift rate obtained from the carbon dioxide in a sample of the NBS Carbon-14 Beta Ray Standard. Known concentrations of the standardized materials in formamide or other solvent were then counted in the cell of figure 2.

Any comparison of counting rates must take into account the density of the solution because the absorption of beta radiation is proportional to the density of the absorbing medium [3, p. 159]. For this reason the counting efficiency (cps/dps) in a given counter should be inversely proportional to the density of the solution counted.

Table 3 presents data for D-mannitol-1-C¹⁴ dissolved in a variety of solvents, and table 4 presents results obtained with a variety of C¹⁴-labeled materials dissolved in the same solvent. The results given in these tables show that the product of the counting efficiency and the density is substantially constant, regardless of the substance counted or

TABLE 2. Reproducibility of successive 8-min counts of D-galactose-1-C¹⁴ in ethylene glycol solution ^a

Proportional counter 1		Proportional counter 2		Proportional counter 3	
Count	Deviation from mean	Count	Deviation from mean	Count	Deviation from mean
97,830	-59	98,227	-183	95,727	+108
98,074	+185	97,894	-516	96,369	+750
98,227	+338	98,458	+47	96,208	+589
97,254	-635	97,754	-656	96,697	+1,078
97,984	+95	98,662	+252	95,806	+187
98,496	+607	98,803	+393	94,994	-625
97,830	-59	98,931	+521	95,120	-499
97,946	+57	98,752	+342	95,394	-225
97,920	+31	98,304	-106	94,950	-669
97,997	+108	98,624	+214	95,813	+194
97,638	-251	98,406	-4	94,678	-941
98,150	+261	98,330	-80	95,299	-320
97,562	-327	98,483	+73	95,503	-116
97,613	-276	97,920	-490	95,569	-50
97,587	-302	98,150	-260	96,147	+528
97,715	-174	98,317	-93	95,511	-108
97,958	+69	97,993	-417	95,646	+27
97,933	+44	98,560	+150	95,366	-253
98,022	+133	98,278	-132	95,698	+79
98,009	+120	98,560	+150	95,523	-96
97,702	-187	98,304	-106	95,848	+239
97,933	+44	98,483	+73	95,220	-399
98,598	+709	98,586	+176	95,796	+177
98,278	+389	98,355	-55	95,397	-222
98,010	+121	98,445	+35	95,819	+200
98,458	+569	98,304	-106	96,030	+411
97,894	+5	98,496	+86	95,440	-179
96,947	-942	98,009	-401	95,758	+139
97,958	+69	98,304	-106	-----	-----
97,920	+31	98,432	+22	-----	-----
97,907	+18	98,854	+444	-----	-----
97,498	-391	98,278	-132	-----	-----
97,331	-558	98,394	-16	-----	-----
97,869	-20	98,778	+368	-----	-----
98,048	+159	99,238	+828	-----	-----
97,920	+31	98,419	+9	-----	-----
98,064	+171	98,227	-183	-----	-----
97,830	-59	98,291	-119	-----	-----
97,779	-110	-----	-----	-----	-----
Avg. 97,889	-----	98,410	-----	95,619	-----
Standard deviation	316	-----	314	-----	409

^a The solution contained 0.424 mg of D-galactose-1-C¹⁴ per milliliter.TABLE 3. Counting efficiency with C¹⁴-labeled D-mannitol in various solvents; correlation of counting efficiency and density

Solvents	Density at 20° C	Counting efficiency	Counting efficiency times density	Calibration factor, μC
				cps \times density
Formamide	1.133	1.000	1.133	2.385 $\times 10^{-3}$
Formamide + 5% H ₂ O ^a	1.127	1.009	1.137	2.377
Formamide + 10% H ₂ O ^a	1.121	1.013	1.136	2.379
Formamide + 20% H ₂ O ^a	1.110	1.025	1.138	2.375
Dimethyl formamide	0.949	1.19	1.129	2.394
Ethylene glycol	1.107	1.02	1.129	2.394
Ethylene glycol + NaI ^b	1.622	0.697	1.131	2.390
Average	-----	-----	1.133	2.385
Standard deviation	-----	-----	0.004	0.007

^a 5, 10, or 20 ml of water diluted to 100 ml with formamide.^b Solution contained 8 g of NaI and 10 ml (11 g) of ethylene glycol.

the solvent. This gives rise to a constant calibration factor that can be used for converting the count of an unknown sample to microcuries of C¹⁴ per milliliter. Thus, for all C¹⁴-labeled materials, the product of cps, density and calibration factor gives microcuries of C¹⁴ per milliliter. The calibration

TABLE 4. Counting efficiency with various C¹⁴-labeled compounds in the same solvents

C ¹⁴ -labeled solute ^a		Density of solution	Disintegration rate ^b per milliliter	Counting rate	Counting efficiency times density	Calibration factor, $\frac{\mu c}{cps \times \text{density}}$
	mg/ml		dps	cps		
D-Mannitol (0.400 μc /mg).....	1.0	1.133	14,800	148.4	1.136	2.379×10^{-3}
D-Mannitol (0.0107 μc /mg).....	25.6	1.139	10,140	100.6	1.130	2.392
D-Mannitol (0.00727 μc /mg).....	55.0	1.145	14,800	146.5	1.133	2.385
D-Mannono- γ -lactone.....	17.4	1.138	2,640	26.4	1.138	2.375
Sodium D- α -glucoheptonate.....	24.1	1.139	5,085	50.4	1.129	2.394
Sodium carbonate ^c	4.0	1.112	18,100	184.4	1.133	2.385
Average.....	-----	-----	-----	-----	1.133	2.385
Standard deviation.....	-----	-----	-----	-----	0.003	0.007

^a All of the materials were dissolved in formamide except the sodium carbonate. This was dissolved in water and an aliquot was diluted 25-fold with ethylene glycol containing 2 percent of sodium hydroxide.

^b Based on the NBS Carbon-14 Beta Ray Standard.

^c To avoid loss of C¹⁴O₂ by CO₂ exchange, the gas for use in the counter was passed through a column of Ascarite.

factor for the instrument used in the present study was 0.002385. This value is characteristic of the instrument and dimensions of the counting cell. It does not vary appreciably from day to day, but major adjustments in the instrument may cause appreciable change. Hence it is necessary to check the calibration from time to time.

The densities of solutions containing small quantities of a solute in a particular solvent do not vary widely. Hence for routine measurements it is not necessary to determine the density of all solutions. Frequently, no substantial error is introduced by use of the density of the solvent for the density of the solution. Density determinations can be avoided by use of a conversion factor obtained by counting a solution of known activity having essentially the same density as that of the unknown. Under these conditions the ratio of microcuries to counts per second obtained for the standard can be used to convert the cps of the unknown to the corresponding microcuries of carbon-14 per milliliter of solution.

The measurement with ethylene glycol containing sodium iodide, reported in table 3, was made to determine whether the presence of an element of high atomic number caused deviations that might arise from backscattering. No such effect was found.

2.6. Applicability of the Method

Counting of carbon-14 in solution is most useful for the assay of highly active samples such as those encountered in synthetic and chemical studies. Although relatively large samples are required, the material may usually be reclaimed by removal of the solvent. The method permits use of volumetric techniques and eliminates the problems inherent in the combustion of samples, in the deposition of films, and in the plating of solids. The method is limited to nonvolatile materials. Even traces of labeled hydrogen cyanide formed by the hydrolysis of cyanide contaminate the counter and must be

avoided. Most of the sugars and sugar derivatives dissolve in formamide and are readily assayed in that solvent. Dimethylformamide is suitable for some materials not soluble in formamide, and ethylene glycol can be used for basic materials that decompose formamide. Thus carbon dioxide can be absorbed in a water solution of either sodium or potassium hydroxide and assayed in aqueous ethylene-glycol solution. In the assay of carbonates the counting gas must be free of carbon dioxide in order to avoid exchange with the labeled carbonate. Alkaline earth carbonates are dissolved in a water solution of the sequestering agent, tetrasodium salt of ethylene diamine tetraacetic acid, and counted after dilution with ethylene glycol.

3. Summary

A simple, rapid, and precise method is presented for the assay of C¹⁴-labeled substances. The material to be assayed is dissolved in a suitable solvent, and the radiation from the solution is measured with a commercial gas-flow proportional counter. The characteristics of suitable solvents are discussed, and it is shown that formamide and ethylene glycol are particularly useful. The counting efficiency was found to be inversely proportional to the density of the solution counted. By counting a substance of known activity under the conditions used for unknown substances, a factor is obtained for converting the count to microcuries of carbon-14 in the sample.

4. References

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